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(57) Abstract

The present invention relates to a competitive lateral flow chromatographic assay device that uses a number of specific binding reactions for determining the presence or amount of an analyte in a test sample. The present device affords greater readability for a user, allowing even inexperienced users to correctly identify positive or negative results. There is less chance of error. The device comprises four serially connected zones with diffusible and attached reagents disposed in at least two of the four zones. There are four main embodiments, each with at least one diffusible reagent, at least one attached specific binding reagent that serves as an analyte test area, and at least one attached specific binding reagent that serves as a device control area. Also disclosed are methods for using such devices and kits employing such devices.

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COMPETITIVE LATERAL FLOW, SPECIFIC BINDING CHROMATOGRAPHIC ASSAY DEVICES, KITS, AND METHODS

Technical Field

The present invention relates to a competitive lateral flow chromatographic assay device that uses a number of specific binding reactions for determining the presence or amount of an analyte in a test sample. The present device affords greater readability for a user, allowing even inexperienced users to identify correctly positive or negative results. There is less chance of error. The device comprises four serially connected zones with diffusible and attached reagents disposed in at least two of the four zones. There are four main embodiments, each with at least one diffusible reagent, at least one attached specific binding reagent that serves as an analyte test area, and at least one attached specific binding reagent that serves as a device control area. Also disclosed are methods for using such devices and kits employing such devices.

Background Art

Immunochromatographic assay devices are known to the art. U.S. 4,168,146 discloses a basic sandwich immunochromatographic assay. A porous support has antibodies covalently bound thereto. An antigen bearing sample is applied to the support and migrates to the reaction site. After a specific binding reaction has occurred, the strip is wet with labeled antibodies that form a sandwich with the bound antibody/ antigen complex. The label can be either a fluorescent component or an enzyme.

U.S. 4,235,601 and U.S. 4,361,537, both to Deutsch et al., disclose a competitive immunochromatographic test device having multiple reaction zones. The first zone is a sample application area. The second zone is a first reagent area wherein the first reagent can move along with sample into a third zone. In the third zone, a second reagent interacts with

the first reagent to form a product which is measured. Competition between the first reagent and the sample reduces the signal. The end of the strip must be immersed into a developing liquid which serves to transport the sample and the first reagent into the reaction zone.

U.S. 4,366,241 to Tom et al. discloses an immunochromatographic test device having a concentrating zone for heterogeneous sandwich immunoassays. An immunosorbing zone characterizes the device. This zone comprises either an antigen or an antibody non-diffusively bound to an area that serves as the inlet port for a bibulous support. First, a sample is deposited in the zone, followed by a solution of a labeled specific binding partner to the non-diffusively bound antigen or antibody.

U.S. 4,435,504 to Syva Co. discloses an immunochromatographic test device having a two enzyme indication system for determining an analyte wherein a specific binding partner and a second enzyme are non-diffusively bound to a bibulous support in a reaction zone. A first enzyme-labeled specific binding partner is applied to the reaction zone along with the sample. The unbound enzyme-labeled partner binds in the reaction zone in relation to the analyte bound in the reaction zone. The first enzyme and second enzyme are related in that the substrate of one is the product from the reaction of another. A signal is generated by applying a substrate.

U.S. 4,446,232 to Liotta discloses an immunochromatographic ELISA test device having two zones with bound antigens or antibodies. The first zone has bound antigens and enzyme labeled antibodies which can react with the bound antigens. The antibodies are positioned within the zone such that they will be removed from the first zone into a second zone if unbound antigens from a sample react, and sample fluids transport the resulting unbound complexes into the second zone, but will not move in the absence of unbound antigens. This means that the antibodies are placed upstream of the bound antigens and react with them. If no antigen is present in the sample, then the bound antigen captures all of the labeled antibodies. The second zone contains enzyme substrate.

U.S. 4,806,311 and U.S. 4,806, 312, both to Greenquist, disclose an immunochromatographic device having multi-zone analytical elements. A labeled reagent with its own detectable property is immobilized in a detection zone without generating a separately migrating species. A reagent zone comprises a solid, porous matrix with an immobilized form of either the analyte, an analog thereof, or the analyte specific binding partner. The labeled reagent may be either applied to the device at the reagent zone or, optionally, two reagent zones may be used, the labeled reagent being deposited in the first reagent zone and the immobilized binding partner in a second. Labeled reagent is complementary to the immobilized component. Thus, if one chooses labeled antibody as the labeled reagent, then the reagent zone has immobilized analyte or analog. On the other hand, if one chooses labeled analyte or analog as the labeled reagent, then the reagent zone has immobilized antibody specific for the analyte or analog. The second zone, (or in some cases third zone), is a detection zone. This zone also has a solid, porous matrix, as well as an immobilized form of a binding substance for the labeled reagent, such as an ion exchange material, or a specific binder, such as immobilized anti-antibody.

U.S. 4,956,302 to Gordon et al. discloses a lateral flow immunochromatographic device. A chromatographic medium has a pre-filtering zone for particulate matter in samples such as with whole blood, a reaction zone, and a downstream zone. The reaction zone has two components therein. The upstream component is a diffusible, labeled specific binding material. The downstream component is an immobilized reagent capable of binding either analyte or the labeled specific binding material. One detects labeled specific binding material at the downstream site in the reaction zone. Upstream from the pre-filtering zone of the medium is a sample application means, either a well or an absorbent pad. Downstream from the medium is a liquid absorption means.

U.S. 5,030,558 to Litman et al. discloses a competitive immunochromatographic assay with a reaction zone separate from the detection zone. A test solution is made containing sample and predetermined amounts of two or more first specific binding partners which are analogous to the analyte and are labeled. This solution is deposited onto a reaction section of bibulous material, on which there already is immobilized predetermined amounts

of two or more second specific binding partners capable of binding either analyte or first specific binding partners. First specific binding partners not bound by the second specific binding partners move downstream and are detected there, increasing with increased analyte.

U.S. 5,104,793 to Buck discloses a multi-zone sandwich immunochromatographic assay and devices therefor. The method requires the use of a three reaction zone strip. The first zone has labeled antibody deposited thereon which can form a complex with the analyte. The second zone contains a mobile inhibitor for the label on the complex. The third zone has a bound capture antibody which forms a sandwich with the labeled complex. The label can be enzymatic or fluorescent. By applying sample, labeled complex and inhibitor are swept into the capture and detection zone. The inhibitor is removed, and a signal is detected.

U.S. 5,156,952 also to Litman et al. discloses a multi-zone competitive immunochromatographic assay and devices therefor which have predetermined threshold analyte levels. A test solution is made containing sample and predetermined amounts of two or more labeled first specific binding partners which are analogous to the analyte. This solution is deposited onto a contact section. Either in the contact section or downstream there is immobilized predetermined amounts of two or more second specific binding partners capable of binding either analyte or first specific binding partners. In the presence of analyte, first specific binding partners not bound by the second specific binding partners move downstream and are detected there, increasing with increased analyte.

U.S. 5,229,073 to Lao et al. discloses a one-step competitive immunochromatographic assay using multiple reaction stripes to quantify lipoprotein analyte amounts. The strip has a depositing zone, with labeled lipoprotein present, and multiple captures zones spaced apart downstream, each with immobilized anti-lipoprotein antibody. The more analyte present, the more stripes having labeled analyte appear.

Disclosure of the Invention

A competitive lateral flow, specific binding chromatographic assay device for determining the presence or amount of an analyte in a test sample comprises four serially connected zones with particular specific binding reagents disposed in particular zones. The present device affords greater readability for a user, allowing even inexperienced users to correctly identify positive or negative results. There is less chance of error. Analytes suitable for the present invention include small organic compounds and other antigenic substances such as herbicides, hormones, microorganisms, nucleic acids, organic compounds, pesticides, proteins, peptides, steroids, therapeutics, toxins, and vitamins. (For the purposes of the present invention, "specific binding reagents" refer to molecules or complexes that specifically bind to another molecule or complex. Thus, the term includes reagents involved in antigen/antibody reactions, biotin/avidin reactions, carbohydrate/lectin reactions, complementary nucleic acid sequence reactions, effector/receptor reactions, enzyme cofactor/enzyme reactions, and the like.)

The first zone is an application zone for receiving a test sample solution. The application zone is comprised of a fluid transport material, such as cellulosics, glass fibers, polyesters, woven or non-woven rayons, as well as bibulous materials such as filter paper. These materials may contain binders for added strength, as known to the art. Reagents that help the release or diffusibility of analyte can be added to this zone. These include blocking agents, sample stabilizers, or solubilizing agents such as proteins, polysaccharides, and surfactants, (such as, anionic, ionic, non-ionic, or zwitterionic surfactants). Typically, blocking agents can aid in the resolubilization and movement of immunoreagents from one zone into another zone within the device. Also, they can discourage non-specific binding of reagents to the chromatographic material in the reaction zone. Blocking agents present in the application zone can move along with analyte or analyte complexes through the chromatographic material. (For the purposes of the present invention, a "fluid transport material" includes materials that support capillary movement of liquids placed thereon, but does not necessarily have a pore structure or surface chemistry that separates proteins chromatographically.)

The second zone is a reagent zone comprised of a fluid transport material having a proximal end and a distal end. The proximal end is connected to the application zone. Diffusible specific binding reagents that participate in competitive specific binding reactions are disposed in this zone. These include labeled conjugates, i.e., a signal component attached to an analyte analog by conventional means, (such as covalent or non-covalent forces), and competitive specific binding reagents (such as antibody/antigens), i.e., a reagent that can form a complex with either the analyte or the labeled conjugate. (Analyte analogs are substances which can also react with a specific binding member for an analyte.) Suitable signal components or labels that are known to the art include, chromagens, colloidal metallic particles, colloidal non-metallic particles, colored latex particles, enzymes, fluorescers, luminescence, stains, and the like.

The third zone is a reaction zone comprised of a chromatographic material, having a proximal end and a distal end. The proximal end is in contact with the distal end of the reagent zone. Typically, chromatographic materials include porous materials capable of transporting the sample solution and reagents from the reagent zone and supporting an irreversible specific binding reaction, either directly or through chemical modifications known to the art. Specific suitable materials include, but are not limited to, cellulosics (such as nitrocellulose), nylon membranes, and polyvinylidene fluoride membranes which are porous, preferably from about 0.05 microns to 15 microns, and wettable, as known to the art. At least two capture reagents are attached to the chromatographic material in the reaction zone. In the test area or line, a first capture reagent can bind specifically either to the analyte, a labeled conjugate, to a complex of a labeled conjugate and a competitive specific binding reagent, or (in some versions of the present invention, if analyte is present in the test sample), to a complex of the competitive specific binding reagent and analyte. The presence of analyte will diminish this signal. In the control area or line, a second capture reagent can bind specifically to the labeled conjugate, at a site different than that of the first capture reagent. This signal tells the user that the competitive reaction occurred, even if no analyte was present. The capture reagents can be disposed in many formats, including being disposed as a series of lateral lines across the advancing fluid front or as a series of non-lateral dots, symbols, *et cetera*, which are equidistant from the sample application zone.

In some embodiments, a third capture reagent is used in a control area or line to aid in interpretation of the assay result. The inclusion of a third capture reagent enables one to incorporate both a positive control line as well as a negative control line into a single assay device. The inclusion of two control lines greatly enhances the readability of the assay by a lay person because one can compare a test result with both a relatively weak and a relatively strong signal, reducing errors in reading the result. The placement of these capture reagents does not have to be in any necessary order, although preferred orders are described in detail in a following section.

The fourth zone in the device is an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone. The bibulous material is capable of drawing solution from the reaction zone. The volume of bibulous material present in the zone is sufficient to draw solution through the chromatographic material in the reaction zone. Suitable bibulous materials include blotting papers, filter papers, non-woven natural polymers, and non-woven synthetic polymers. The dimensions of the materials will vary depending upon sample size and absorptive capacity, as known to the art.

The present invention including these four zones can be modified to include elements known to the art, such as pre-filters and sample flow control layers.

The general method for using the present specific binding chromatographic assay device is as follows. First, a test sample solution is applied to an application zone which contains buffer and a wetting agent, as described above. The volume of the sample solution is sufficient to wet the application zone, a reagent zone, a reaction zone, and be drawn into an absorption zone for at least one minute, the four zones being serially connected for fluid transport. Typically, these times will range from one minute to twenty minutes. Samples having a thicker viscosity, such as blood, will take longer times than more aqueous solutions. Sufficient time is allowed to pass for the sample solution to traverse the reagent zone and the reaction zone, and go into the absorption zone, all as described above. The solution is drawn from the chromatographic material in the reaction zone, typically for at least one minute.

The reaction zone produces a first detectable signal related to the presence or amount of analyte. In some cases, as with enzyme systems, a solution containing enzyme substrate must be added to the reaction zone in order to create the detectable signal. The control area produces a second detectable signal, (and in some cases, a third detectable signal), which indicates a successful operation of the device, regardless of the presence of analyte.

A kit for determining the presence or amount of an analyte in a test sample comprises the specific binding chromatographic assay device as described above and a wetting solution which can be applied to the application zone of the specific binding chromatographic device, either alone or in combination with the test sample. (For the purposes of the present invention, "wetting solutions" include solutions which can extract or resolubilize an analyte from a sample source.) This wetting solution is capable of wetting the application zone, the reagent zone, the reaction zone, and being absorbed into the absorption zone. Typically, suitable wetting solutions include aqueous buffered solutions suitable for physiological samples as well as organic solvents for the extraction of non-aqueous samples.

Suitable analytes for the present invention may be found in physiological fluids, environmental fluids, soil samples, food processing fluids, or foodstuffs. Physiological fluids include blood, lymphatic fluids, saliva, spinal fluid, sweat, urine, and the like. Of course, conventional processing of these fluids may be needed to prepare a sample. As known to the ordinarily skilled artisan, the sample may require filtration, extraction, or concentration. In addition, interfering components present in the sample may need to be removed or inactivated.

Brief Description of the Drawings

FIGURE 1 is a isometric view of a preferred embodiment of the device illustrating the housing.

FIGURE 2 is an overhead view of the device with the upper portion of the housing removed.

FIGURE 3 is a diagrammatic view of a preferred embodiment of the present invention using one diffusible reagent and three reaction lines.

FIGURE 4 is an isometric view of both positive and negative results from the assay formats shown in FIGURES 3 and 5.

FIGURE 5 is a diagrammatic view of a preferred embodiment of the present invention using two diffusible reagents and three reaction lines.

FIGURE 6 is a diagrammatic view of a first and a second preferred embodiment of the present invention using two diffusible reagents and two reaction lines.

FIGURE 7 is an isometric view of both positive and negative results from the assay format shown in FIGURE 6.

Best Modes for Carrying Out the Invention

Preferred embodiments of the present invention comprise enzyme-linked, competitive, multi-zone, lateral flow immunoassays that use horseradish peroxidase (HRP). The assays are performed on a lateral flow strip (10) having four zones -- a sample application zone (12), a reagent zone (14), an indicating reaction zone (16), and an excess absorbency zone (18). The zones can be assembled onto a common structural backing material (20) that does not readily allow lateral flow of a solution (such as ARcare 7823, made by Adhesives Research, Inc. of Glen Rock, Pennsylvania, U. S. A.). As seen in Figures 1 and 2, this assembly can then be placed inside a structural housing (22) having a first port (24) over the application zone which forms a well for placing the sample, and a second port (26) over the reaction zone so as to allow the user to view the signal from reagents thereon.

Typically, this device can be quite small, having a laminating strip of about 6 cm by 20 cm. Alternatively, the assembly can be exposed for use as a dipstick.

The sample application zone is comprised of a glass fiber impregnated with a Tris buffered, (pH 7.5), saline solution (TBS) containing a surfactant, such as Triton® X-100 (Rohm & Haas), (0.01%-0.5%(w/v)), deposited thereon. (Preferably, this glass fiber is No. 9254, made by Lydall, Inc. of Rochester, New Hampshire, U. S. A.). Distal to this first zone is the reagent deposit zone, also comprised of glass fiber, but not having any surfactant or TBS present. Diffusible reagents are deposited on or about the glass fiber. These reagents may be placed on the glass fiber through conventional dispensing means, preferably as a lateral line having an even concentration of reagent. Following the deposited reagent zone is the indicating reaction zone. Preferably this zone is comprised of a nitrocellulose paper (NC) having a pore size of about 3 to 15 microns, (preferably SSLU™ made by Schleicher & Schuell, Inc. of Dassel, Germany), backed by a plastic for structural support. Capture reagents are attached or immobilized here. These reagents can be either antibodies which bind to diffusible reagents, analyte, or complexes of both. The most distal zone, the excess absorbency zone, is comprised of an absorbent material, such as 900 cellulose paper, (made by Schleicher & Schuell, Inc. of Keene, New Hampshire, U.S.A.).

The following examples of preferred embodiments of the present invention all involve assays for atrazine, a triazine pesticide, as an analyte. The atrazine sample can come from water or be absorbed onto non-liquid materials, such as food stuffs, soil, or packaging. Obviously, in the case of non-liquid materials conventional sample processing which is known to those of ordinary skill in the art must occur in order to solubilize any atrazine which may be present. However, the ordinarily skilled artisan can also appreciate that the present invention is applicable to many analytes, as described above.

FIRST EMBODIMENT

A first version of the present invention having one diffusible reagent and three reaction lines is illustrated in Figure 3.

A diffusible reagent (32) comprised of an atrazine-horseradish peroxidase conjugate (AHRP) solution, is applied laterally across the reagent zone as a stripe. The AHRP solution comprises 0.01 to 10 ug/ml, (preferably 8 ug/ml), of an AHRP conjugate, in a pH 7.5 Tris buffered solution also containing 0.05% to 10% (w/v), (preferably 1%) bovine serum albumen (BSA), 0.5% to 10% (w/v), (preferably 5%), sucrose, and 0.5% to 50% (w/v), (preferably 30%), trehalose.

Three capture reagents are deposited, all laterally, in the reaction zone. A first capture reagent (34) is a rabbit anti-atrazine antibody (RAAA) solution in phosphate buffered saline (PBS), pH 7.2. (The RAAA solution can be made with an antibody available from Biostride, Inc. of Palo Alto, California.) Downstream from the first line is a second line. This second line comprises a second capture reagent (36) which is an anti-HRP (A-HRP) solution in PBS, pH 7.2. (The A-HRP solution can be made with a polyclonal goat antibody from Jackson ImmunoResearch Laboratories, Inc. of West Grove, Pennsylvania, U. S. A.) This second capture reagent is distal to the first capture line by a distance that allows a clear differentiation between signal readouts when read by the user and to provide for no interference between reactions, about 5 mm. The binding capacity of the deposited second capture reagent is balanced so as to capture enough signal component to produce a readable signal at the threshold limit for analyte detection for the assay. While typically for atrazine this would be about 3 ppb, one can adjust the readout to detect either greater or lesser threshold amounts. Finally, a third capture reagent (38) is also an A-HRP solution similar to the second capture reagent except that the concentration is higher so as to result in the capture of more signal molecule. The binding capacity of the deposited third capture reagent is balanced so as to be able to capture enough signal component to produce a readable signal that substantially matches the readable signal produced by the first capture reagent when no analyte is present in the sample. This third capture reagent is distal to the second capture line by about 2 mm. All three capture reagents are attached to the nitrocellulose through the particular protein binding surface characteristics of nitrocellulose. If another chromatographic material was selected, then, in certain cases known to the art, conventional immobilization techniques may be needed.

Four drops (0.15 to 0.20 ml) of sample (30) are added to the application zone, in these examples it is an aqueous solution having from 0 to 20 ng/ml of atrazine (A). As the sample moves into the reagent zone, the AHRP is solubilized into the sample solution. Within about 5 to 7 seconds after sample application, the solution is swept into the reaction zone. The AHRP and A compete to bind to the RAAA in the first line. The amount of AHRP deposited in the reagent zone is sufficient such that if no A is present in the sample, then there is excess AHRP that can sweep past the first line and react and bind with both the second capture reagent and the third capture reagent. The second and third lines in the reaction zone serve as end of assay indicators. After a specified time, (with HRP as the signal component the time is about five minutes to twenty minutes), an HRP substrate such as a tetramethyl benzidine (TMB) solution is added to the strip by means of dispensing into the viewing port (26).

Color will develop at all three lines. A true negative result is shown in Figure 4. Here, at the first capture reagent (34) a competition reaction occurs, unlike at the second and third capture reagents (36 and 38). Because of this competition reaction, the intensity of this first line will vary, unlike the intensities of the more distal lines. For example, in a negative result, the first capture reagent has more AHRP than A bound to it, making the color intensity closer to that produced by the combination of the third capture reagent and A-HRP than to the color intensity produced by the combination of the second capture reagent and A-HRP. (Color intensity is indicated by the width of the lines.) A true positive result also is shown in Figure 4. Now, the first and second lines are approximately the same in color intensity, (indicated by the width of the lines), while the third line is noticeably more intense. (In this assay, increasing the amount of analyte present in the sample diminishes the intensity of color development in the first line.) The readability of the assay is enhanced by the comparative function. The user simply has to decide whether the first line looks closer to the second or the third line. With the present elements, this assay can detect less than 1.0 ng of atrazine.

SECOND EMBODIMENT

A second version of the present invention having two diffusible reagents and three reaction lines is illustrated in Figure 5.

A first diffusible reagent (31) comprised of an RAAA solution, is impregnated laterally across the reagent zone as a first stripe. The RAAA solution comprises 0.01 mg/ml to 10 mg/ml of RAAA in a PBS solution, pH 7.5, also containing about 5% to 25%, (preferably 20%), w/v glycerol and about 0.5% to 10%, (preferably 5%), w/v sucrose. This solution is applied to the reagent zone at a defined rate, usually dispensing a final volume of 0.1 ul/cm to 10 ul/cm. A second diffusible reagent (32) comprised of an AHRP solution as described above, is applied laterally across the reagent zone as a second stripe, distal to the first stripe by about 3 mm.

Three capture reagents are deposited, all laterally, in the reaction zone. A first capture reagent (34) is a goat anti-rabbit Ig (GARIG) solution containing about 0.01 mg/ml to 10 mg/ml of GARIG in a PBS solution, pH 7.2. (The GARIG solution is made with an antibody from Schleicher & Schuell, Inc. of Keene, New Hampshire, U.S.A.). A second line is downstream. This second line comprises a second capture reagent (36) is an A-HRP solution similar to that described above. This second capture reagent is distal to the first capture line by a distance that allows a clear differentiation between signal readouts when read by the user and provides for no interference between reactions, about 5 mm.. The binding capacity of the deposited second capture reagent is balanced so as to capture enough signal component to produce a readable signal at the threshold limit for analyte detection for the assay. While typically for atrazine this would be about 3 ppb, one can adjust the readout to detect either greater or lesser threshold amounts. Finally, a third capture reagent (38) is also an A-HRP solution similar to the second capture reagent except that the concentration is higher so as to result in the capture of more signal molecule. The binding capacity of the deposited third capture reagent is balanced so as to be able to capture enough signal component so as to produce a readable signal that substantially matches the readable signal produced by the first capture reagent when no analyte is present in the sample. This third

capture reagent is distal to the second capture line by about 2 mm. All three capture reagents are attached to the nitrocellulose through the particular protein binding surface characteristics of nitrocellulose. If another chromatographic material was selected, then, in certain cases known to the art, conventional immobilization techniques may be needed.

Four drops (0.15 ml to 0.20 ml) of sample (30) are added to the application zone, in these examples it is an aqueous solution having 0 ng/ml to 20 ng/ml of atrazine (A). As the sample moves into the reagent zone, the RAAA and the AHRP are solubilized into the sample solution. A competition starts between AHRP and A for binding with the RAAA so as to form either RAAA-A complex or RAAA-AHRP complex. Within about 5 to 7 seconds after sample application, the solution is swept into the reaction zone. These complexes bind to the GARIG in the first line. The amount of AHRP deposited in the reagent zone is sufficient such that if no A is present in the sample, then there is excess AHRP complex that can sweep past the first line and react and bind with both the second capture reagent and the third capture reagent. The second and third lines in the reaction zone serve as end of assay indicators. After a specified time, TMB is added to the strip as drops into the viewing port (26).

Color will develop at all three lines. A true negative result is shown in Figure 4. Here, at the first capture reagent (34) a competition reaction occurs, unlike at the second and third capture reagents (36 and 38). Because of this competition reaction, the intensity of this first line will vary, unlike the intensities of the more distal lines. For example, in a negative result, the first capture reagent has more RAAA-AHRP complex than RAAA-A bound to it, making the color intensity closer to that produced by the combination of the third capture reagent and A-HRP than to the color intensity produced by the combination of the second capture reagent and A-HRP. (Color intensity is indicated by the width of the lines.) A true positive result also is shown in Figure 4. Now, the first and second lines are approximately the same in color intensity, (indicated by the width of the lines), while the third line is noticeably more intense. (In this assay, increasing the amount of analyte present in the sample intensifies the end-of-assay line and diminishes the intensity of color development in the first line.) The readability of the assay is enhanced by the comparative function. The

user simply has to decide whether the first line looks closer to the second or the third line.

THIRD EMBODIMENT

A first version of the present invention having two diffusible reagents and two reaction lines is illustrated in Figure 6.

A first diffusible reagent (31) comprised of an RAAA solution, as described above, is impregnated laterally across the reagent zone as a first stripe. A second diffusible reagent (32) comprised of an AHRP solution as described above, is impregnated laterally across the reagent zone as a second stripe distal to the first stripe.

Two capture reagents are deposited, all laterally, in the reaction zone. A first capture reagent (34) is a goat anti-rabbit Ig (GARIG) solution as described above. Downstream from the first line is a second line. This second line comprises a second capture reagent (36) which is an A-HRP solution similar to that described above. This second capture reagent is distal to the first capture line by a distance that allows a clear differentiation between signal readouts when read by the user and provides for no interference between reactions, about 5 mm. The binding capacity of the deposited second capture reagent is balanced so as to capture enough signal component to produce a readable signal that substantially matches the readable signal produced by the first capture reagent when no analyte is present. Both capture reagents are attached to the nitrocellulose through the particular protein binding surface characteristics of nitrocellulose. If another chromatographic material was selected, then, in certain cases known to the art, conventional immobilization techniques may be needed.

Four drops (0.15 ml to 0.20 ml) of sample (30) are added to the application zone, in these examples it is an aqueous solution having 0 ng/ml to 20 ng/ml of atrazine (A). As the sample moves into the reagent zone, the RAAA and the AHRP are solubilized into the sample solution. A competition starts between AHRP and A for binding with the RAAA so as to form either RAAA-A complex or RAAA-AHRP complex. Within about 5 to 7 seconds

after sample application, the solution is swept into the reaction zone. These complexes bind to the GARIG in the first line. The amount of AHRP deposited in the reagent zone is sufficient such that if no A is present in the sample, then there is excess AHRP complex that can sweep past the first line and react and bind with the second capture reagent. The second line in the reaction zone serves as an end of assay indicator. After a specified time, TMB is added to the strip as drops into the viewing port (26).

Color will develop at both lines. A true negative result is shown in Figure 7. Here, at the first capture reagent (34) a competition reaction occurs, unlike at the second reagent (36). Because of this competition reaction, the intensity of this first line will vary, unlike the intensity of the more distal line. For example, in a negative result, the first capture reagent has more RAAA-AHRP complex than RAAA-A bound to it. (Color intensity is indicated by the width of the lines.) Here, the first and second lines are approximately the same in color intensity, (indicated by the width of the lines). A true positive result also is shown in Figure 7. Now, the first and second lines are of noticeably different color intensities, (indicated by the width of the lines), the second line being noticeably more intense.

FOURTH EMBODIMENT

A second version of the present invention having two diffusible reagents and two reaction lines is illustrated in Figure 8.

A first diffusible reagent (31) comprised of an RAAA solution, as described above, is impregnated laterally across the reagent zone as a first stripe. A second diffusible reagent (32) comprised of an AHRP solution as described above, is impregnated laterally across the reagent zone as a second stripe.

Two capture reagents are deposited, all laterally, in the reaction zone. A first capture reagent (34) is a goat anti-rabbit Ig (GARIG) solution as described above. Downstream from the first line is a second line. This second line comprises a second capture reagent (36)

which is an RAAA solution similar to that described above. This second capture reagent is distal to the first capture line by a distance that allows a clear differentiation between signal readouts when read by the user and provides for no interference between reactions, about 5 mm. The binding capacity of the deposited second capture reagent is balanced so as to capture enough signal component to produce a readable signal that substantially matches the readable signal produced by the first capture reagent when no analyte is present. Both capture reagents are attached to the nitrocellulose through the particular protein binding surface characteristics of nitrocellulose. If another chromatographic material was selected, then, in certain cases known to the art, conventional immobilization techniques may be needed.

Four drops (0.15 ml to 0.20 ml) of sample (30) are added to the application zone, in these examples it is an aqueous solution having 0 to 20 ng/ml of atrazine (A). As the sample moves into the reagent zone, the RAAA and the AHRP are solubilized into the sample solution. A competition starts between AHRP and A for binding with the RAAA so as to form either RAAA-A complex or RAAA-AHRP complex. Within about 5 to 7 seconds after sample application, the solution is swept into the reaction zone. These complexes bind to the GARIG in the first line. The amount of AHRP deposited in the reagent zone is sufficient such that if no A is present in the sample, then there is excess AHRP complex that can sweep past the first line and react and bind with the second capture reagent. The second line in the reaction zone serves as an end of assay indicator. After a specified time, TMB is added to the strip as drops into the viewing port (26).

Color will develop at both lines. A true negative result is shown in Figure 7. Here, at the first capture reagent (34) a competition reaction occurs, unlike at the second reagent (36). Because of this competition reaction, the intensity of this first line will vary, unlike the intensity of the more distal line. For example, in a negative result, the first capture reagent has more RAAA-AHRP complex than RAAA-A bound to it. (Color intensity is indicated by the width of the lines.) Here, the first and second lines are approximately the same in color intensity, (indicated by the width of the lines). A true positive result also is shown in Figure 7. Now, the first and second lines are of noticeably different color intensities, (indicated by

the width of the lines), the second line being noticeably more intense. (In this assay, increasing the amount of analyte present in the sample intensifies the end-of-assay line and diminishes the intensity of color development in the first line.)

The placement of the capture reagents can be changed, and the present assays are still functional. For example, one can have the following sequence of capture reagents – A-HRP/RAAA/A-HRP as opposed to the RAAA/A-HRP/A-HRP sequence. In this case, a negative readout would have a less intense line most proximal, followed by two equally more intense lines. A positive readout would also start with a less intense line, followed by a second less intense line, then a more intense, most distal line.

The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.

CLAIMS:

1. A competitive lateral flow, specific binding chromatographic assay device for determining the presence or amount of an analyte in a test sample comprising:
   a) an application zone for receiving a test sample, said application zone being comprised of a fluid transport material;
   b) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;
   c) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;
   d) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;
   e) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the labeled conjugate or the analyte;
   f) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the labeled conjugate;
   g) a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to bind specifically to the labeled conjugate; and
   h) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone.

2. The device of Claim 1 wherein blocking agents are disposed about the fluid transport material in the application zone.

3. The device of Claim 2 wherein the blocking agent is a surfactant.

4. The device of Claim 1 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

5. The device of Claim 1 wherein the first, second, and third capture reagents are disposed laterally across the reaction zone as separate lines, the second capture reagent is distal to the first capture reagent and the third capture reagent is distal to the second capture reagent.

6. The device in Claim 1 wherein the second capture reagent and the third capture reagent are the same.

7. The device in Claim 1 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

8. A competitive lateral flow, specific binding chromatographic assay device for determining the presence or amount of an analyte in a test sample comprising:

a) an application zone for receiving a test sample, said application zone being comprised of a fluid transport material;

b) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

c) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

d) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

e) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

f) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the competitive

specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

g) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the labeled conjugate;

h) a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to bind specifically to the labeled conjugate; and

i) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone.

9. The device of Claim 8 wherein the competitive specific binding reagent is disposed proximally to the application zone with respect to the labeled conjugate

10. The device of Claim 8 wherein blocking agents are disposed about the fluid transport material in the application zone.

11. The device of Claim 10 wherein the blocking agent is a surfactant.

12. The device of Claim 8 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

13. The device of Claim 8 wherein the first, second, and third capture reagents are disposed laterally across the reaction zone as separate lines, the second capture reagent is distal to the first capture reagent and the third capture reagent is distal to the second capture reagent.

14. The device in Claim 8 wherein the second capture reagent and the third capture reagent are the same.

15. The device in Claim 8 wherein the volume of bibulous material is sufficient to draw

solution from the chromatographic material in the reaction zone for at least one minute.

16. A competitive lateral flow, specific binding chromatographic assay device for determining the presence or amount of an analyte in a test sample comprising:

a) an application zone for receiving a test sample, said application zone being comprised of a fluid transport material;

b) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

c) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

d) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

e) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

f) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

g) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the signal component of the labeled conjugate without affecting the ability of the signal component to produce a signal; and

h) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone.

17. The device of Claim 16 wherein the competitive specific binding reagent is disposed

proximally to the application zone with respect to the labeled conjugate.

18. The device of Claim 16 wherein blocking agents are disposed about the fluid transport material in the application zone.

19. The device of Claim 18 wherein the blocking agent is a surfactant.

20. The device of Claim 16 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

21. The device of Claim 16 wherein the first and second capture reagents are disposed laterally across the reaction zone as separate lines, and the second capture reagent is distal to the first capture reagent.

22. The device in Claim 16 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

23. A competitive lateral flow, specific binding chromatographic assay device for determining the presence or amount of an analyte in a test sample comprising:

a) an application zone for receiving a test sample, said application zone being comprised of a fluid transport material;

b) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

c) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

d) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

e) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of

a chromatographic material;

f) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

g) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically either to the analyte analog portion of the labeled conjugate or to analyte; and

h) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone.

24. The device of Claim 23 wherein the competitive specific binding reagent is disposed proximally to the application zone with respect to the labeled conjugate.

25. The device of Claim 23 wherein blocking agents are disposed about the fluid transport material in the application zone.

26. The device of Claim 25 wherein the blocking agent is a surfactant.

27. The device of Claim 23 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

28. The device of Claim 23 wherein the first and second capture reagents are disposed laterally across the reaction zone as separate lines, and the second capture reagent is distal to the first capture reagent.

29. The device in Claim 23 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

30. A method for determining the presence or amount of an analyte in a test sample using a competitive lateral flow, specific binding chromatographic assay device comprising:

a) applying a sample solution to an application zone which is comprised of a fluid transport material, the volume of the sample solution being sufficient to wet the application zone, a reagent zone, a reaction zone, and to be drawn into an absorption zone for at least one minute, the four zones being serially connected for fluid transport;

b) allowing sufficient time to pass for the sample solution to traverse the reagent zone which is comprised of a fluid transport material having a distal end and a proximal end, said proximal end being connected to the application zone, said reagent zone having a diffusible labeled conjugate, comprised of a signal component attached to an analyte analog, disposed about the fluid transport material in the reagent zone;

c) allowing sufficient time to pass for the sample solution to traverse the reaction zone which has a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, said reaction zone being comprised of a chromatographic material and having a first capture reagent attached to the chromatographic material, said first capture reagent being able to bind specifically with either the labeled conjugate or the analyte, a second capture reagent attached to the chromatographic material in the reaction, said second capture reagent being able to bind specifically to the labeled conjugate, and a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to bind specifically to the labeled conjugate;

d) allowing sufficient time to pass for the sample solution to traverse from the reaction zone and into the absorption zone which is comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone, whereby the first capture reagent can produce a first detectable signal related to the presence or amount of analyte, the second capture reagent can produce a second detectable signal for control purposes which indicates a successful operation of the device and provides a signal intensity

comparable to the signal intensity of the first detectable signal when analyte is present at a detectable level, and the third capture reagent can produce a third detectable signal for control purposes which indicates a successful operation of the device and provides a signal intensity comparable to the signal intensity of the first detectable signal when analyte is not present at a detectable level; and

e) observing the first detectable signal, the second detectable signal, and the third detectable signal.

31. The method of Claim 30 wherein blocking agents are disposed about the fluid transport material in the application zone.

32. The method of Claim 30 wherein the blocking agent is a surfactant.

33. The method of Claim 31 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

34. The method of Claim 30 wherein the first, second, and third capture reagents are disposed laterally across the reaction zone as separate lines, the second capture reagent is distal to the first capture reagent, and the third capture reagent is distal to the second capture reagent.

35. The method in Claim 30 wherein the second capture reagent and the third capture reagent are the same.

36. The method in Claim 30 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute

37. A method for determining the presence or amount of an analyte in a test sample using a competitive lateral flow, specific binding chromatographic assay device comprising:

a) applying a sample solution to an application zone which is comprised of a fluid transport material, the volume of the sample solution being sufficient to wet the

application zone, a reagent zone, a reaction zone, and to be drawn into an absorption zone for at least one minute, the four zones being serially connected for fluid transport;

b) allowing sufficient time to pass for the sample solution to traverse the reagent zone which is comprised of a fluid transport material having a distal end and a proximal end, said proximal end being connected to the application zone, said reagent zone having a diffusible labeled conjugate, comprised of a signal component attached to an analyte analog, disposed about the fluid transport material in the reagent zone, and a diffusible competitive specific binding reagent also disposed about the fluid transport material;

c) allowing sufficient time to pass for the sample solution to traverse the reaction zone which has a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, said reaction zone being comprised of a chromatographic material and having a first capture reagent attached to the chromatographic material, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte, a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the labeled conjugate, and a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to bind specifically to the labeled conjugate;

d) allowing sufficient time to pass for the sample solution to traverse from the reaction zone and into the absorption zone which is comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone, whereby the first capture reagent can produce a first detectable signal related to the presence or amount of analyte, the second capture reagent can produce a second detectable signal for control purposes which indicates a successful operation of the device and provides a signal intensity

comparable to the signal intensity of the first detectable signal when analyte is present at a detectable level, and the third capture reagent can produce a third detectable signal for control purposes which indicates a successful operation of the device and provides a signal intensity comparable to the signal intensity of the first detectable signal when analyte is not present at a detectable level; and

e) observing the first detectable signal, the second detectable signal, and the third detectable signal.

38. The method of Claim 37 wherein the competitive specific binding reagent is disposed proximally to the application zone with respect to the labeled conjugate.

39. The method of Claim 37 wherein blocking agents are disposed about the fluid transport material in the application zone.

40. The method of Claim 39 wherein the blocking agent is a surfactant.

41. The method of Claim 37 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

42. The method of Claim 37 wherein the first, second, and third capture reagents are disposed laterally across the reaction zone as separate lines, the second capture reagent is distal to the first capture reagent, and the third capture reagent is distal to the second capture reagent..

43. The method in Claim 37 wherein the second capture reagent and the third capture reagent are the same.

44. The method in Claim 37 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

45. A method for determining the presence or amount of an analyte in a test sample using a

competitive lateral flow, specific binding chromatographic assay device comprising:

a) applying a sample solution to an application zone which is comprised of a fluid transport material, the volume of the sample solution being sufficient to wet the application zone, a reagent zone, a reaction zone, and to be drawn into an absorption zone for at least one minute, the four zones being serially connected for fluid transport;

b) allowing sufficient time to pass for the sample solution to traverse the reagent zone which is comprised of a fluid transport material having a distal end and a proximal end, said proximal end being connected to the application zone, said reagent zone having a diffusible labeled conjugate, comprised of a signal component attached to an analyte analog, disposed about the fluid transport material in the reagent zone, and a diffusible competitive specific binding reagent also disposed about the fluid transport material;

c) allowing sufficient time to pass for the sample solution to traverse the reaction zone which has a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, said reaction zone being comprised of a chromatographic material and having a first capture reagent attached to the chromatographic material, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte, and a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the signal component of the labeled conjugate;

d) allowing sufficient time to pass for the sample solution to traverse from the reaction zone and into the absorption zone which is comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone, whereby the first capture reagent can produce a first detectable signal related to the presence or amount of analyte, the second capture reagent can produce a second detectable signal for control purposes which

indicates a successful operation of the device; and

e) observing the first detectable signal, and the second detectable signal.

46. The method of Claim 45 wherein the competitive specific binding reagent is disposed proximally to the application zone with respect to the labeled conjugate.

47. The method of Claim 45 wherein blocking agents are disposed about the fluid transport material in the application zone.

48. The method of Claim 47 wherein the blocking agent is a surfactant.

49. The method of Claim 45 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

50. The method of Claim 45 wherein the first and second capture reagents are disposed laterally across the reaction zone as separate lines, and the second capture reagent is distal to the first capture reagent.

51. The method in Claim 45 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

52. A method for determining the presence or amount of an analyte in a test sample using a competitive lateral flow, specific binding chromatographic assay device comprising:

a) applying a sample solution to an application zone which is comprised of a fluid transport material, the volume of the sample solution being sufficient to wet the application zone, a reagent zone, a reaction zone, and to be drawn into an absorption zone for at least one minute, the four zones being serially connected for fluid transport;

b) allowing sufficient time to pass for the sample solution to traverse the reagent zone which is comprised of a fluid transport material having a distal end and a proximal end, said proximal end being connected to the application zone, said reagent zone

having a diffusible labeled conjugate, comprised of a signal component attached to an analyte analog, disposed about the fluid transport material in the reagent zone, and a diffusible competitive specific binding reagent also disposed about the fluid transport material;

c) allowing sufficient time to pass for the sample solution to traverse the reaction zone which has a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, said reaction zone being comprised of a chromatographic material and having a first capture reagent attached to the chromatographic material, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte, and a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically either to the analyte analog portion of the labeled conjugate or to analyte;

d) allowing sufficient time to pass for the sample solution to traverse from the reaction zone and into the absorption zone which is comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone, whereby the first capture reagent can produce a first detectable signal related to the presence or amount of analyte, the second capture reagent can produce a second detectable signal for control purposes which indicates a successful operation of the device; and

e) observing the first detectable signal, and the second detectable signal.

53. The method of Claim 52 wherein the competitive specific binding reagent is disposed proximally to the application zone with respect to the labeled conjugate.

54. The method of Claim 52 wherein blocking agents are disposed about the fluid transport material in the application zone.

55. The method of Claim 54 wherein the blocking agent is a surfactant.

56. The method of Claim 52 wherein the diffusible labeled conjugate is disposed laterally across the reagent zone as a line.

57. The method of Claim 52 wherein the first and second capture reagents are disposed laterally across the reaction zone as separate lines, and the second capture reagent is distal to the first capture reagent.

58. The method in Claim 52 wherein the volume of bibulous material is sufficient to draw solution from the chromatographic material in the reaction zone for at least one minute.

59. A kit for determining the presence or amount of an analyte in a test sample comprising:

a) a competitive lateral flow, specific binding chromatographic assay device comprising:

i) an application zone for receiving a test sample and a wetting solution, said application zone being comprised of a fluid transport material;

ii) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

iii) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

iv) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

v) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the labeled conjugate or the analyte;

vi) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the labeled conjugate;

vii) a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to specifically bind to the labeled conjugate; and

viii) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone; and

b) a wetting solution which can be applied to the application zone of the specific binding chromatographic device either alone or in combination with the test sample, said wetting solution being capable of wetting the application zone, the reagent zone, the reaction zone, and being absorbed into the absorption zone.

60. A kit for determining the presence or amount of an analyte in a test sample comprising:

a) a competitive lateral flow, specific binding chromatographic assay device comprising:

i) an application zone for receiving a test sample and a wetting solution, said application zone being comprised of a fluid transport material;

ii) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

iii) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

iv) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

v) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

vi) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the

competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

vii) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the labeled conjugate;

viii) a third capture reagent attached to the chromatographic material in the reaction zone, said third capture reagent being able to bind specifically to the labeled conjugate; and

ix) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone; and

b) a wetting solution which can be applied to the application zone of the specific binding chromatographic device either alone or in combination with the test sample, said wetting solution being capable of wetting the application zone, the reagent zone, the reaction zone, and being absorbed into the absorption zone.

61. A kit for determining the presence or amount of an analyte in a test sample comprising:

a) a competitive lateral flow, specific binding chromatographic assay device comprising:

i) an application zone for receiving a test sample and a wetting solution, said application zone being comprised of a fluid transport material;

ii) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

iii) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

iv) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

v) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

vi) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

vii) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the signal component of the labeled conjugate without affecting the ability of the signal component to produce a signal; and

viii) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone; and

b) a wetting solution which can be applied to the application zone of the specific binding chromatographic device either alone or in combination with the test sample, said wetting solution being capable of wetting the application zone, the reagent zone, the reaction zone, and being absorbed into the absorption zone.

62. A kit for determining the presence or amount of an analyte in a test sample comprising:

a) a competitive lateral flow, specific binding chromatographic assay device comprising:

i) an application zone for receiving a test sample and a wetting solution, said application zone being comprised of a fluid transport material;

ii) a reagent zone comprised of a fluid transport material having a distal end and a proximal end, said proximal end being in contact with the application zone;

iii) a diffusible labeled conjugate comprised of a signal component attached to an analyte analog, said labeled conjugate being disposed about the fluid transport material in the reagent zone;

iv) a diffusible competitive specific binding reagent being disposed about the reagent zone and being able to bind specifically with either the labeled conjugate or the analyte;

v) a reaction zone having a distal end and a proximal end, said proximal end being in contact with the distal end of the reagent zone, the reaction zone being comprised of a chromatographic material;

vi) a first capture reagent attached to the chromatographic material in the reaction zone, said first capture reagent being able to bind specifically with either the competitive specific binding reagent or a complex of the competitive specific binding reagent and either the labeled conjugate or the analyte;

vii) a second capture reagent attached to the chromatographic material in the reaction zone, said second capture reagent being able to bind specifically to the analyte analog portion of the labeled conjugate; and

viii) an absorption zone comprised of a bibulous material that is in contact with the distal end of the reaction zone, said bibulous material being capable of drawing solution from the reaction zone, the volume of bibulous material being sufficient to draw solution from the chromatographic material in the reaction zone; and

b) a wetting solution which can be applied to the application zone of the specific binding chromatographic device either alone or in combination with the test sample, said wetting solution being capable of wetting the application zone, the reagent zone, the reaction zone, and being absorbed into the absorption zone.

FIGURE 1

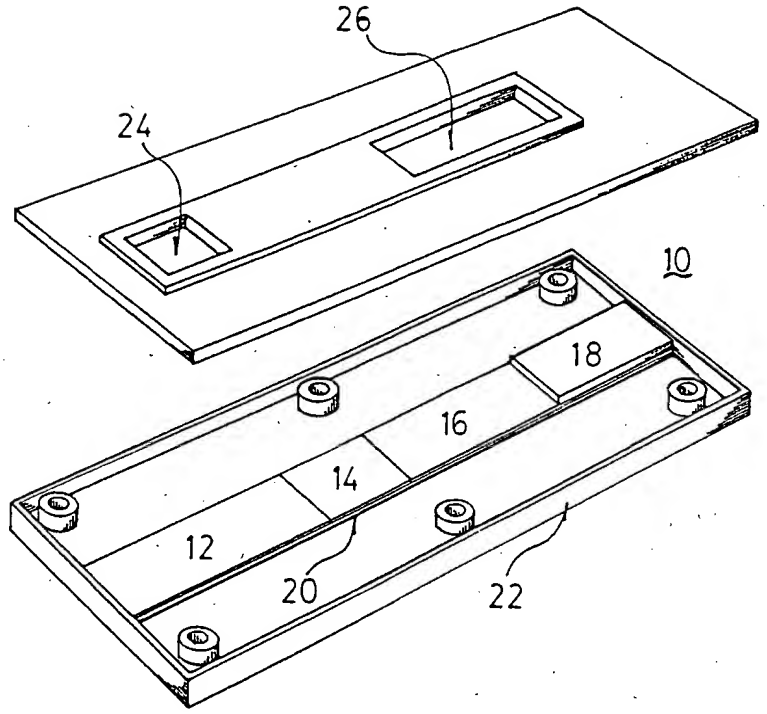


FIGURE 2

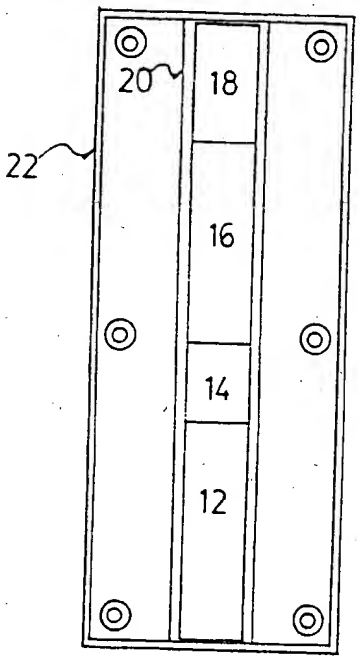


FIGURE 3

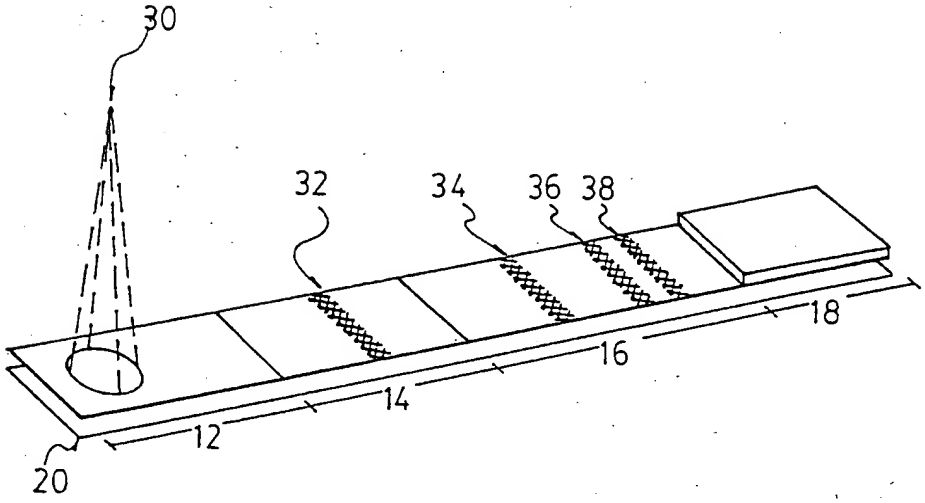
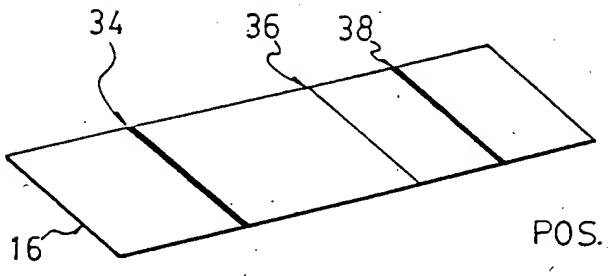


FIGURE 4



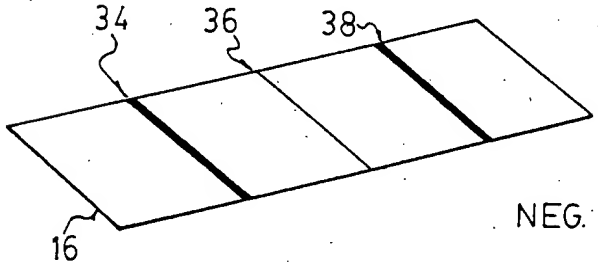


FIGURE 5

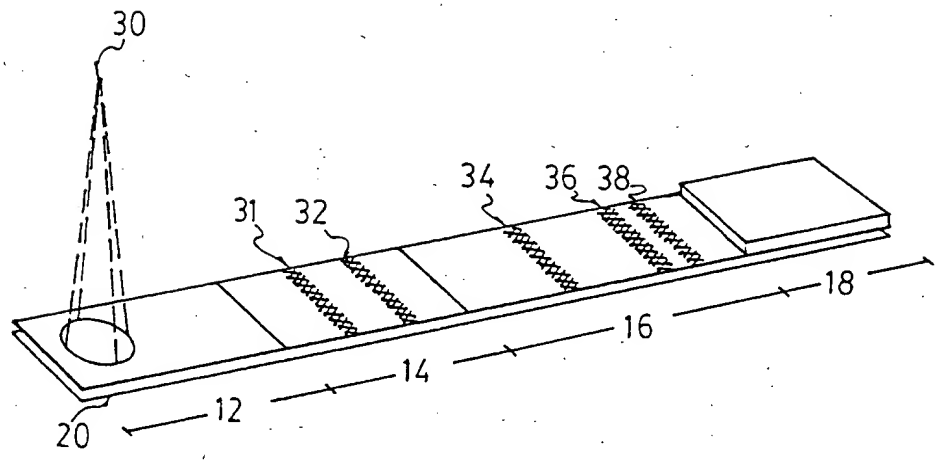


FIGURE 6

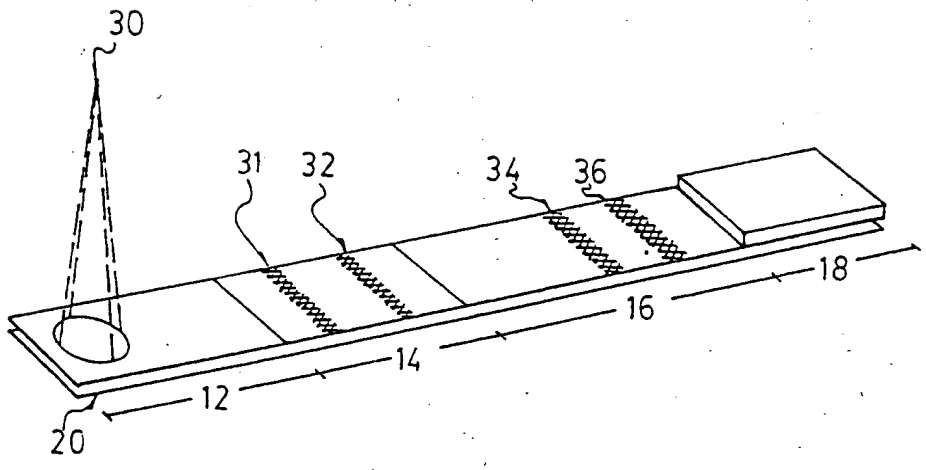
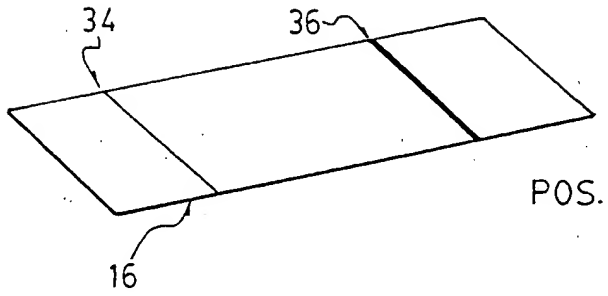
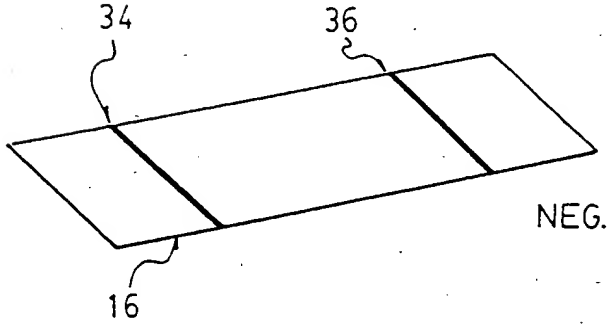


FIGURE 7





INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21831

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/558
US CL :436/514
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/56, 57, 58; 435/7.5, 7.93, 287.2, 287.7, 287.9, 810, 970, 975; 436/514, 518, 531, 807, 808, 810

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

None

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 4,959,307 A (OLSON) 25 September 1990, see entire document, especially column 3, lines 29-58 and 65-67; column 8, lines 29-36; column 8, line 58 to column 9, line 2; column 11, lines 58-62; column 12, lines 21-28; and column 15, lines 28-30.	1-3, 5-8, 10-11, 14-16, 18-19, 22-23, 25-26, 29, 59-62 ---------- 4, 9, 12, 17, 20, 24, 27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 FEBRUARY 1998	Date of mailing of the international search report 13 MAR 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Susan C. Wolski Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21831

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,156,953 A (LITMAN et al.) 20 October 1992, see entire document, especially column 3, lines 17-23; column 4, lines 1-40; column 8, lines 14-34; column 9, lines 1-19; column 11, lines 18-27; column 12, lines 39-40; column 13, line 59 to column 14, line 9; and column 14, lines 38-51.	1-62